

Defective Interfering Particles of Poliovirus

I. Isolation and Physical Properties

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A class of defective interfering (DI) poliovirus particles has been identified. The first was found as a contaminant of a viral stock; others have been isolated by serial passage at a high multiplicity of infection. The DI particles are less dense than standard virus and sediment more slowly. Their ribonucleic acid (RNA) sediments more slowly than standard RNA and has a higher electrophoretic mobility. Competition hybridization experiments with double-stranded viral RNA indicate that DI RNA is 80 to 90% of the length of standard RNA. The proteins of DI particles are indistinguishable from those of standard poliovirus.

Defective interfering (DI) particles are found in stocks of many different types of viruses (9). These particles are a natural form of mutant which could be useful in the study of viral multiplication. They are also of interest because of their possible role in viral disease processes (9). In our stocks of type 1 poliovirus, what appears to be a DI particle has been found. Two other DI particles have been isolated by repeated passage of clonally purified poliovirus at a high multiplicity of infection. The original DI has been named DI(1) and the others, DI(2) and DI(3).

In this paper we will report the isolation and physical characterization of poliovirus DI particles. In a subsequent paper (*in preparation*) it will be shown that the DI particles can initiate all aspects of the poliovirus replication cycle except for the manufacture of new DI particles because they are apparently lacking some of the genes for capsid proteins. They can be propagated by the use of helper, standard poliovirus, but this co-infection results in interference by the DI particles with the production of standard poliovirus.

MATERIALS AND METHODS

Virus and cells. The growth of suspended HeLa cells to a concentration of 4×10^5 /ml and their infection by type 1 poliovirus have been described previously (2).

Poliovirus plaque assay. A modified Cooper-type (5) plaque assay was used. For the base layer equal amounts of 2% Agar (Difco) at 55 C and twofold concentrated medium plus 5% horse serum were mixed. The mixture was kept at 45 C and 8-ml portions were dispensed into 60 by 15 mm bacterial plastic petri dishes. Plates were stored at 4 C for up to 3 months before further use.

The following were prepared for the top layer: (i) autoclaved 1.2% agar in medium, kept at 60 C;

(ii) virus diluted serially in medium; (iii) HeLa cells washed twice with medium and resuspended at 10^7 /ml in medium plus 1% horse serum. A mixture of 0.2 ml of a given virus dilution with 0.6 ml of cells and 0.6 ml of agar were spread rapidly over base layer plates at room temperature, and plates were incubated at 37 C in a CO₂ incubator. After 40 to 48 hr, 2.0 ml of a solution of 0.01% Neutral Red in Earle's saline was added to each plate; stain was removed after 2 to 4 hr at 37 C and plaques were made more visible by addition of 0.5 ml of glacial acetic acid to each plate (C. Pfau, *personal communication*).

Isolation of poliovirus DI strains by repeated serial passage. A single plaque on a plate containing fewer than 10 plaques was picked into 1.0-ml medium using a capillary tube. This was immediately cloned and two new plaques, from different plates with fewer than six plaques, were selected. Each plaque was used to produce a clonally purified virus stock by infecting 4×10^6 cells with virus from the selected plaque. The infection was allowed to continue approximately 72 hr until all cells had been lysed, with addition of fresh medium after 24 hr to maintain a cell concentration between 20×10^4 and 40×10^4 /ml.

Successive passages at a high multiplicity of infection were then carried out in two parallel series, A and B, derived from the two separate plaques. For the first passage infection was carried out at a multiplicity of 5 plaque-forming units per cell for A and 15 for B. The multiplicity of infection was increased through the first five passages and from then on the multiplicity of infection was always greater than 100 plaque-forming units per cell.

Production of labeled virus. From 4×10^7 to 4×10^8 cells were washed once in Earle's saline (6) and infected by resuspension at more than $100\times$ normal concentration in medium containing about 20 plaque-forming units of virus per cell. Either plaque-purified (DI free) stocks of poliovirus or a stock containing approximately equal amounts of standard poliovirus and DI particles was used. After adsorption at room temperature for 15 to 30 min, cells were diluted to

4×10^6 /ml with warm medium containing horse serum (5%) and actinomycin D (5 μ g/ml) and incubated at 37 C. Radioactive label was added 90 min later.

To label proteins, cells were washed once in Earle's saline, resuspended in medium minus leucine containing dialyzed horse serum (5%) and 3 H-leucine (10 to 20 μ Ci/ml) or 14 C-leucine (0.5 to 2.0 μ Ci/ml). Ribonucleic acid (RNA) was labeled with uridine or radiophosphate. 14 C-uridine (0.5 to 1.0 μ Ci/ml) or 3 H-uridine (10 to 20 μ Ci/ml) was added directly to the infected cells; 10 mCi of carrier-free radio-phosphate (in 1.1 ml of 0.15 M NaCl) was added to 4×10^7 infected cells which had been washed once in medium lacking phosphate and resuspended at 4×10^6 /ml in medium lacking phosphate containing 5% dialyzed horse serum.

Purification of virus on CsCl gradients. Labeled virus was released from cells by three cycles of freezing and thawing followed by removal of cellular debris by centrifugation. Sodium dodecyl sulfate was added to 0.5% to the virus-containing supernatant and virus was harvested by centrifugation at 160,000 $\times g$ for 90 min in the type 65 rotor in an L2-65B ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). Large volumes of fluid were harvested at 78,000 $\times g$ for 2.5 hr in the type 30 rotor.

In infections of more than 4×10^8 cells or when 32 PO₄ was used, the harvested virus was resuspended in 0.5 ml of RS buffer (0.01 M tris(hydroxymethyl) aminomethane (Tris), pH 7.4; 0.01 M NaCl; 1.5×10^{-3} M MgCl₂), layered over a 36-ml, linear 15 to 30% sucrose gradient in 0.5% sodium dodecyl sulfate buffer (0.1 M NaCl; 0.01 M Tris, pH 7.5; 10^{-3} M ethylenediaminetetraacetic acid (EDTA); 0.5% sodium dodecyl sulfate), and centrifuged at 22 C for 3 hr at 81,500 $\times g$ in the SW27 rotor. The gradient was collected into 1-ml fractions through a recording Gilford spectrophotometer. Fractions containing virus as determined by optical density at 260 nm, OD₂₆₀, were pooled and diluted with 0.5% sodium dodecyl sulfate buffer, and the virus was harvested as above in the type 65 rotor.

The virus was resuspended in 1 ml of NE buffer (0.01 M Tris, pH 7.4; 0.01 M NaCl; 0.02 M EDTA) and transferred to cellulose nitrate tubes (1.59 by 7.6 cm; $\frac{5}{8}$ by 3 inches). The necessary components were added to make a 6-ml solution of CsCl (density = 1.33 g/cc), 0.8% Brij-58, and NE buffer, and this was overlaid with Nujol. Ultracentrifugation was carried out in type 40 or type 65 rotors at 93,000 $\times g$ for 14 hr at 4 C in the Beckman model L or L2-65B ultracentrifuge. Gradients were collected by puncturing the tubes at the bottom. Radioactivity in the fractions was analyzed as previously described (8). Densities were determined by refractometry. Preparations containing unlabeled virus were analyzed by diluting fractions to 0.4 ml with NE buffer and measuring OD₂₆₀ with a Gilford spectrophotometer.

Preparation of poliovirus RNA. RNA was extracted from purified virions by using either the phenol extraction method of Scherrer and Darnell (18) or the sodium dodecyl sulfate-acetic acid extraction method (7, 13). Both methods yielded RNA with identical

electrophoretic behavior. The detergent method was utilized for preparation of material for acrylamide gels and the phenol method for preparation of material for hybridization experiments in order to remove all protein.

Preparation of poliovirus double-stranded RNA. Poliovirus double-stranded RNA was prepared by a modification of the method of Bishop and Koch (3). After ethanol precipitation the RNA was dissolved in 0.02 M sodium phosphate, pH 7.2, at a concentration of 1 to 2 mg/ml, made 2 M in LiCl, and placed at -20 C overnight. Single-stranded and partially double-stranded RNA were removed by centrifugation for 10 min at 12,000 $\times g$ in a Sorvall RC2B centrifuge. Double-stranded RNA, which remained in the supernatant (1), was precipitated with ethanol, collected by centrifugation in the Sorvall RC2B centrifuge, dissolved in 1.0 ml of 0.5% sodium dodecyl sulfate buffer, and layered over a 14-ml, linear 15 to 30% sucrose gradient in the same buffer. Gradients were centrifuged at 20 C for 18 hr at 120,000 $\times g$ in the Beckman SW 40 rotor. Half-milliliter fractions were collected through the Gilford spectrophotometer and the fractions containing the peak of 18S poliovirus double-stranded RNA (1), seen by its absorption at 260 nm, were pooled. By this procedure 2×10^9 cells yielded 2.2 OD₂₆₀ of poliovirus double-stranded RNA. A parallel preparation of labeled double-stranded RNA prepared from 8×10^7 cells and labeled with 400 μ Ci of 3 H-uridine yielded 2.5×10^6 counts per min of 3 H-uridine-labeled, double-stranded poliovirus RNA.

Acrylamide gel electrophoresis. To separate proteins, acrylamide gels were prepared and electrophoresed by a modification (11, 16) of the method of Summers, Maizel, and Darnell (21). Gels 6 cm in length were prepared in plexiglass tubes (7.5-cm) having a 0.84 cm ($\frac{1}{4}$ inch) inside diameter; 12-cm gels were prepared in tubes (15 cm). After electrophoresis, gels were removed from tubes and sliced into 1-mm slices (21); slices were prepared for liquid scintillation counting (4). Additional electrophoresis experiments were performed by the method of Maizel and Laemmli (*personal communication*). Electrophoresis of RNA was performed by the method of Loening (12) as modified by Weinberg et al. (22).

Hybridization experiments. Poliovirus standard RNA was obtained by extraction of RNA from a preparation of poliovirus made from a stock free of DI contamination. Pure DI(1) RNA was obtained by extraction of RNA from the DI(1) region of the final purification on CsCl resulting from an infection using a mixed poliovirus and DI(1) stock. The purification is such that the pooled DI(1) virions have less than 1% of the specific infectivity (plaque-forming units per OD₂₆₀) of standard poliovirus.

Annealing of poliovirus standard and DI(1) RNA to poliovirus double-stranded RNA utilized methods previously described (10) except that denaturation of RNA was carried out by boiling in water rather than a high salt buffer.

Materials. 14 C-L-leucine (200 mCi/mole), 3 H-L-leucine (2 to 10 Ci/mole), uridine-2- 14 C (50 mCi/mole), and uridine-5- 3 H (20 Ci/mole) were

purchased from New England Nuclear Corp., Des Plaines, Ill. $^{32}\text{PO}_4$ as phosphoric acid was purchased from New England Nuclear Corp.; it was made 0.15 M in NaCl and then adjusted to pH 7.4 by the addition of a small amount of 0.5 M Tris buffer (pH 7.5).

Ribonuclease A was purchased from Worthington Biochemical Corp., Freehold, N.J.; ribonuclease T₁ (B grade) was purchased from Calbiochem, Los Angeles, Calif. Actinomycin D was a generous gift from Merck Sharp and Dohme. Sodium dodecyl sulfate was obtained from Matheson, Coleman, and Bell; Ficoll from Pharmacia, Inc.; CsCl from Harshaw Chemical Co.; and iodoacetamide and Cleland's reagent from Sigma Chemical Co., St. Louis, Mo.

RESULTS

Appearance and isolation of a DI particle from poliovirus. Sedimentation of poliovirus in a sucrose gradient normally produces a single symmetrical band at about 150S. However, when a preparation of poliovirus which was prepared after a large number of serial passages over several years was purified by sedimentation in a sucrose gradient, material sedimenting more slowly than poliovirus was observed as a shoulder on the virion peak (Fig. 1a).

In order to characterize the slower sedimenting

material, samples were taken from fractions in two regions (b and c) of Fig. 1a and the viral particles in each sedimented to equilibrium in CsCl. Two distinct bands were observed in each gradient, one at the density of poliovirus (1.34 g/cc; reference 14) and a second at a lower density (about 1.325 g/cc). Region b (Fig. 1b) contained predominantly standard poliovirus, whereas the slower sedimenting material (region c) contained a greater proportion of lower density material (Fig. 1c). Throughout the course of the following experiments, the absolute densities of the material in the two bands, as measured by refractometry, varied by as much as 0.01 g/cc, but the density difference between the two bands was always 0.015 to 0.02 g/cc. Experiments to be presented below and in a subsequent paper (*manuscript in preparation*) have shown that the material of density 1.325 is a DI form of poliovirus, analogous to the DI particles associated with many other types of viruses (9). Because a number of poliovirus DI particles have now been isolated, we refer to the initial isolate as DI(1).

Isolation of additional defective particles. Two additional DI particles, DI(2) and DI(3), have been isolated. Repeated passages at a high multiplicity of infection using plaque-purified virus were performed in two parallel series (A and B) as described above. At selected passage numbers, the particle yield was labeled with ^3H -uridine and examined in CsCl density gradients. In series A only a single, symmetrical peak was visible in passages 10, 12, or 16 (Fig. 2). There was a slight suggestion of lower density material as a shoulder on the major band at passage 17 or 18, but it was not until passage 19 that a prominent shoulder became visible. By passage 21 a distinct band of lighter particles was apparent, and by passage 25 the two bands were of approximately equal magnitude. During passage series B a shoulder first appeared in passage 20 and a bimodal distribution was observed in passage 25. The lighter particles derived from series A and B have been named DI(2) and DI(3), respectively. None of the DI particles which have been isolated is necessarily pure because no method for cloning the DI particles has been developed.

Both DI(2) and DI(3) differed from standard poliovirus by a larger density than did DI(1). The differences observed were 0.028 g/cc for DI(2) and 0.026 g/cc for DI(3). To compare directly the density of DI(1) and DI(3), progeny virus from the 25th passage of series B, containing both standard and DI(3) virions labeled with ^{14}C -uridine, was mixed with a small amount of pure ^3H -uridine labeled DI(1). Centrifugation of the mixture to equilibrium in CsCl showed that

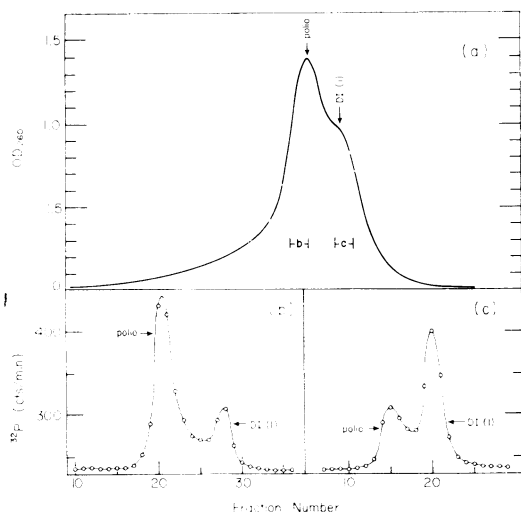


FIG. 1. Sucrose gradient pattern and buoyant densities of a laboratory stock of poliovirus particles. (a) OD_{260} profile of the bottom half of a sucrose gradient containing a preparation of ^{32}P -labeled poliovirus. Sedimentation was from right to left. (b) and (c) Samples of fractions 11 (b) and 14 (c) were mixed with 3 g of CsCl, 0.13 ml of 10% Brij-58 detergent, and RS buffer to form a 7-ml gradient, centrifuged for 28 hr at $78,000 \times g$ at 4°C in an SW-40 rotor (Beckman, Instruments, Inc.), collected into 0.2-ml fractions, and analyzed for radioactivity. Higher density is at the left.

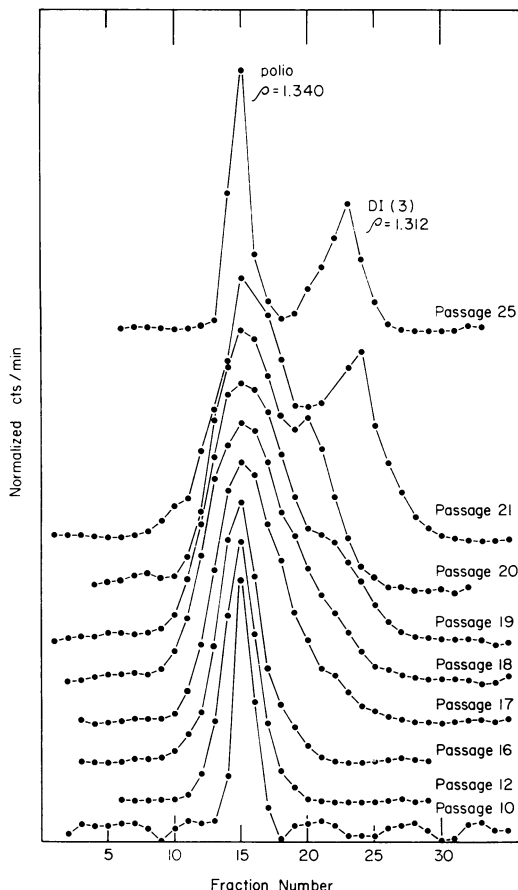


FIG 2. Appearance of DI particles during successive passages of clonally purified poliovirus. CsCl gradients containing the labeled progeny of the indicated passages were prepared as described in the text. The maximum amplitude of the standard poliovirus band has been assigned a value of 1 and the remainder of each gradient normalized to this value. No adjustment has been made to account for different numbers of fractions produced by different gradients, and therefore no precise comparison of poliovirus band-width or standard and DI band separation can be made. Indicated densities are for passage 25 virus only.

DI(3) was lighter than DI(1) (Fig. 3). A similar result was obtained when DI(2) was compared to DI(1).

Comparison of the RNA of standard and DI virions. The RNA of standard and DI virions were compared both by velocity sedimentation in sucrose and by acrylamide gel electrophoresis. ^{14}C -uridine-labeled standard RNA and ^3H -uridine-labeled DI(1) RNA were sedimented together through a sucrose gradient. The RNA of DI(1) sedimented more slowly than that of standard virions (Fig. 4a), whereas in the control differ-

entially-labeled standard RNA sedimented at identical rates (Fig. 4b).

Comparison of standard and DI(1) RNA by acrylamide gel electrophoresis indicated that the mobility of DI(1) RNA was greater than that of standard RNA (Fig. 5a). The RNA of DI(3) migrated slightly faster than that of DI(1) (Fig. 5b).

These experiments suggest that DI(1) RNA is shorter than standard poliovirus RNA and that DI(3) RNA is even shorter than DI(1) RNA. The latter result is consistent with the finding that DI(3) virions have a lower density than those of DI(1).

Comparison of DI(1) and standard RNA by hybridization. Table 1 shows that both standard and DI(1) RNA are able to convert about 50% of denatured labeled double-stranded poliovirus RNA to a ribonuclease-resistant form. This result demonstrates that DI(1) RNA is homologous to base sequence found in poliovirus double-stranded RNA. However, because of a $\pm 5\%$ variability in such experiments and their high background, a different type of experiment was necessary in order to determine the exact size of the DI(1) RNA and also to determine to which strand of the double-stranded RNA it is complementary.

In order to obtain a more exact measure of the difference in size of standard and DI(1) RNA and to determine whether standard poliovirus and DI RNA share sequences or are complementary, competition hybridization experiments were conducted. Assays were performed using constant amounts of denatured unlabeled poliovirus double-stranded RNA and ^{32}P -labeled poliovirus

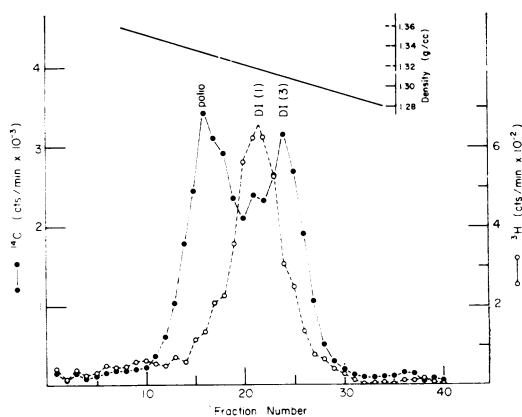


FIG. 3. Comparison of buoyant densities of DI(1) and DI(3) in CsCl. Gradients were prepared and centrifuged as described. Symbols: (●), ^{14}C -uridine-labeled progeny of the 25th passage of series B; (○), ^3H -uridine-labeled DI(3).

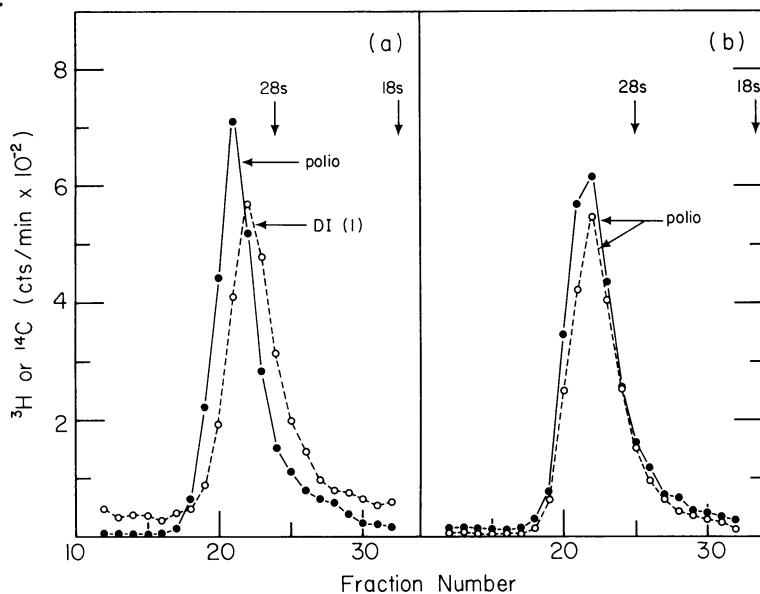


FIG. 4. Comparison of the sedimentation rates of standard and DI(1) RNA in sucrose gradients. Labeled RNA was extracted by the acetic acid-sodium dodecyl sulfate method, precipitated by the addition of 2 volumes of 95% ethanol plus 50 μg of carrier yeast transfer RNA per ml, collected by centrifugation at $12,000 \times g$ in the RC2B Sorvall centrifuge, resuspended in 0.5 ml of 0.5% sodium dodecyl sulfate buffer, layered over a 16-ml linear 15 to 30% sucrose gradient in the same buffer, and sedimented for 15.5 hr at $66,000 \times g$ in the SW-27 rotor (Beckman Instruments Inc.). (a) ^{14}C -uridine-labeled standard RNA and ^3H -uridine-labeled DI(1) RNA; (b) ^{14}C - and ^3H -uridine-labeled standard RNA.

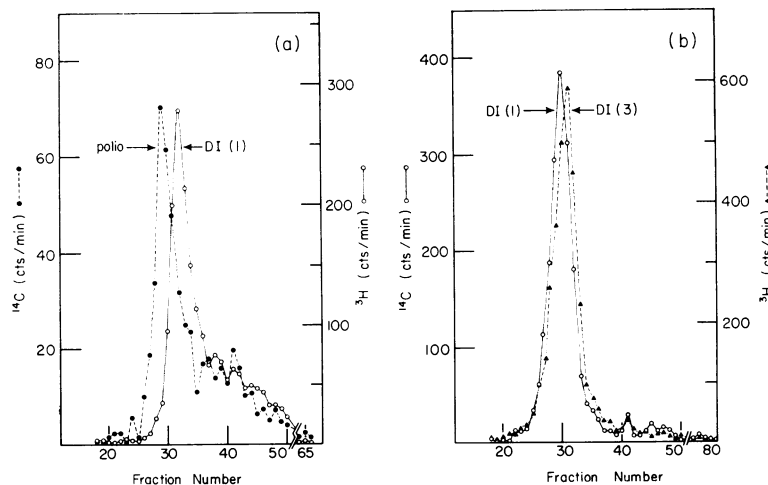


FIG. 5. Comparison of the electrophoretic mobility of standard, DI(1) and DI(3) RNA in polyacrylamide gels. Phenol-extracted standard, DI(1) and DI(3) RNA were ethanol precipitated and ether extracted. Samples were resuspended in 50 μl of electrophoresis sample buffer [0.04 M tris(hydroxymethyl)aminomethane, pH 7.4; 0.02 M sodium acetate; 2×10^{-3} M ethylenediaminetetraacetic acid; 0.5% sodium dodecyl sulfate] containing sucrose and methylene blue (21) and samples were mixed for co-electrophoresis. Up to 50 μl of mixed sample was layered on to 6-cm 2.6% acrylamide gels which had been prerun 15 min at 5 ma/gel, and electrophoresis was carried out at 5 ma/gel for 4 to 4.5 hr. Gels were frozen, removed from the tubes, sliced, and assayed for radioactivity. The anode is at the right.

TABLE 1. Annealing of unlabeled standard and defective interfering [DI(1)] ribonucleic acid (RNA) to ^{14}C -double-stranded standard RNA^a

Conditions	Ribo- nuclease- resistance (%)
Not boiled.....	99
Boiled, not annealed.....	9.7
Boiled.....	8.3
Boiled, standard RNA added.....	52.3
Boiled, DI(1) RNA added.....	50.9

^a Mixtures were made containing about 1,200 counts/min of poliovirus double-stranded RNA and either 7.5 μg of standard poliovirus RNA or no added RNA in a final volume of 0.4 ml of H_2O . Indicated samples were boiled 5 min. Samples were annealed at 70 C for 45 min after adjustment to 0.1% sodium dodecyl sulfate and 0.3 M NaCl and 0.03 M sodium citrate. Samples were chilled and divided into two equal portions, one of which was treated with 50 μg of ribonuclease A per ml for 30 min at 22 C. All samples were then acid precipitated and radioactivity was determined. The results are expressed as percentage of ribonuclease-resistant RNA in each sample.

RNA of high specific activity ($\sim 1.2 \times 10^6$ counts per min per μg). In parallel experiments increasing amounts of unlabeled standard or DI(1) RNA were added to the above reagents in order to determine the extent to which the added unlabeled RNA could compete for annealing with the ^{32}P -labeled standard RNA. The results of several experiments are summarized in Fig. 6. Both standard and DI(1) RNA competed with ^{32}P -labeled standard RNA during the annealing reaction, indicating that DI(1) RNA consists mainly of base sequences identical to those found in standard RNA. However, as more RNA was added a constant difference of about 13% was seen between the two competition curves. This suggests that 13% of the standard RNA is lacking in DI(1) RNA. DI(1) RNA, therefore, consists of 87% of the poliovirus genome. The data in Fig. 5 indicate that DI(3) RNA is slightly smaller than DI(1) RNA; we estimate it to contain 83% of the genome, based on its electrophoretic mobility.

Comparison of the capsid proteins of standard and DI particles. The proteins comprising the capsids of DI(1) and DI(3) were compared to those of poliovirus by electrophoresis on 10% acrylamide gels (Fig. 7a and b). The same four capsid proteins were present in both standard and DI particles and in the same relative proportions.

DISCUSSION

When clonally purified poliovirus is repeatedly passaged at high multiplicity in HeLa cells, there eventually appear particles of lower buoyant density. In two cases such particles were first observed in CsCl gradients after the 16th and 18th passages, respectively. They had previously been observed as a contaminant of one laboratory stock of poliovirus. We know of no other report

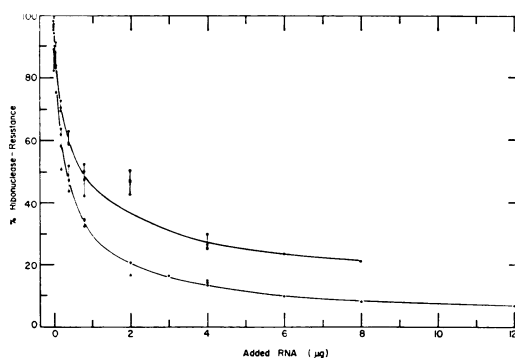


FIG. 6. Determination of the size of DI(1) RNA by competition hybridization. Conditions described below were determined by preliminary experiments for optimal time, temperature, and concentration of reagents needed to give complete double-stranded RNA denaturation and maximal renaturation to added RNA. Hybridization was performed in dichromate-washed tubes in a final volume of 0.2 ml of H_2O containing 0.4 μg of poliovirus double-stranded RNA, 1,500 counts/min of ^{32}P -labeled RNA (approximately 0.004 μg of RNA), and increasing amounts of unlabeled standard or DI(1) RNA. Samples were boiled for 5 min, transferred to a water bath at 70 C, and made 0.1% in sodium dodecyl sulfate and 2X SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate). Annealing was for 2 hr. Samples were then rapidly chilled and divided into two equal portions, one set acid precipitated and the other set treated with 50 μg of ribonuclease A per ml and 25 units of ribonuclease T_1 per ml for 30 min at 22 C before acid precipitation. Both sets of samples were collected on membrane filters (Millipore Corp., Bedford, Mass.) and radioactivity determined by counting in a gas flow counter (Nuclear-Chicago Corp.) The figure contains data from several experiments. Symbols: (●), standard RNA; (○), DI(1) RNA. Percentage of ribonuclease-resistance was calculated for each sample, and 2.1% was subtracted to account for ribonuclease-resistant material present in the ^{32}P -labeled RNA and not denatured upon treatment at 100 C for 5 min. The data were then adjusted so that 100% was defined as the level of ribonuclease-resistance obtained after maximally annealing ^{32}P -labeled RNA to poliovirus double-stranded RNA in the absence of added, competing single-stranded RNA. This value was actually 86%.

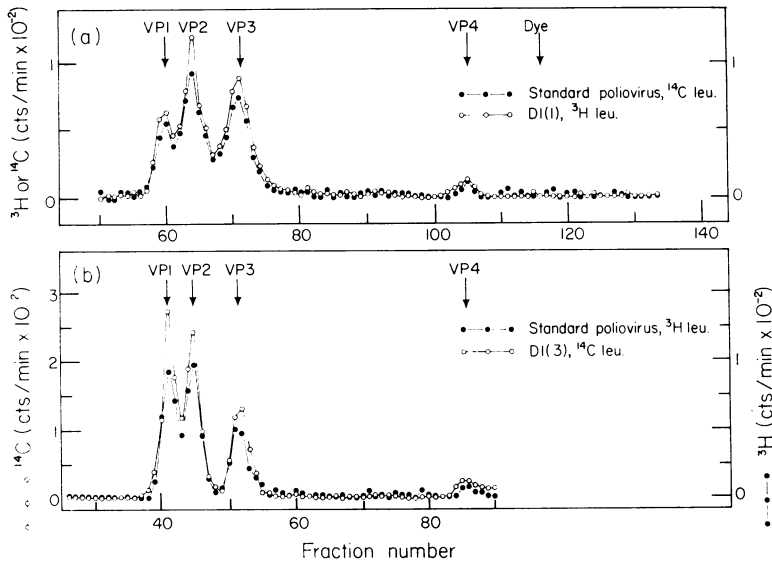


FIG. 7. Comparison of the capsid proteins of standard, DI(1) and DI(3) particles by polyacrylamide gel electrophoresis. (a) Standard and DI(1) particles were purified on CsCl gradients. Samples were prepared and electrophoresis carried out by a modification (11, 16) of the method of Summers, Maizel, and Darnell (21). One hundred μ liters of sample (containing standard and DI(1) capsid proteins) was layered onto a 12-cm 10% acrylamide-ethylene diacrylate gel and electrophoresis carried out at 7 ma/gel for 18 hr. (b) Standard and DI(3) particles were purified on CsCl gradients and the particles removed from the CsCl by dilution with distilled water and centrifugation at $160,000 \times g$ at 4 C for 60 min in the Beckman 65 rotor. Viral particles were resuspended in distilled water, mixed with electrophoresis sample buffer, and boiled for 5 min. A sample of 0.3 ml of this mixture was layered onto 10% acrylamide-bis-acrylamide gels, and electrophoresis carried out at 100 v/gel for 5 hr by the method of Maizel and Laemmli (personal communication). After electrophoresis, gels (a) and (b) were removed from tubes and sliced, and radioactivity was assayed.

of picornavirus DI particles although they may have been present in earlier experiments (17).

The need for 16 to 18 successive high multiplicity passages to generate detectable levels of poliovirus DI particles is in contrast to the appearance of DI particles after three or four such passages from vesicular stomatitis virus (19) and reovirus (15). This might reflect the rarity of DI production in the poliovirus system. More likely, it is due to the weaker interference of poliovirus DI, as compared to other DI, which lengthens the time between the initial production of DI particles and growth to a titer where they can be detected by our methods.

The analyses reported here show that the DI particles consist of a portion of the poliovirus genome plus a capsid made of all four poliovirus coat proteins in proportions identical to those for standard poliovirus. It is probable that these particles are formed by replication of the defective RNA by a mechanism similar or identical to that by which standard RNA is replicated and that this defective RNA is then encapsidated by the poliovirus procapsid. It has been shown (11, 20) that the procapsid contains three proteins, VP 0,

VP 1, and VP 3, and that VP 0 is apparently converted to VP 2 and VP 4 upon union of the procapsid with viral RNA. We can detect no VP 0 in our DI particles (Fig. 7) so it seems that the conversion of VP 0 to VP 2 and VP 4 is complete even though a reduced amount of RNA is present.

One aspect of the results in Fig. 6 should be noted. The hybridization competition by DI(1) RNA has a single point off the line at 2 μ g of added RNA and this has been a reproducible result. It may indicate that the population of DI(1) RNA molecules has some type of heterogeneity in spite of the sharp profile exhibited by the particles in CsCl gradients and the apparent homogeneity of the RNA on sucrose gradients and in acrylamide gel electrophoresis. Standard RNA does not show this deviation from a smooth curve. In spite of this unresolved oddity, the difference between the competition by standard and DI RNA at high RNA inputs clearly indicates that about 13% of the standard RNA is not represented in DI(1); but the exact genetic structure of the DI RNA can only be determined from more detailed experiments, and homogeneity

will only be assured if a method of cloning DI particles can be devised.

In order to justify the designation of the poliovirus-related particles discussed in this paper as DI particles, it is necessary to show that they interfere with the growth of standard virus. Evidence for interference will be presented in a subsequent paper.

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